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Early Diagnosis of Leukemia by Microarray Analysis for Ameliorate Suffering and Reducing Mortality of Patients

Harendra Modak[1], Sujayendra S. Kulkarni[1], Suyamindra S. Kulkarni[2], Pramod B.Gai[1,2]

[1] Centre for Excellence in Molecular Haemato-oncology, Dept. of Applied Genetics, Karnatak University, Dharwad

[2] Karnataka Institute for DNA Research, Pavate Nagar, Dharwar-580003

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Abstract

Introduction:

This research analyzes the patternsof the relative frequency for occurrence rate of leukemia in North Karnataka region and identification of candidate genes and novel mutations responsible for hematologic malignanciesto provide more specific treatment leading towards minimization of the suffering of patients.

Material and Methods:

The detailed reports of 230 females as well as 417 males (F:M ratio 1:1.8) were collected from different hospitals of North Karnataka focusing on variables like lifestyle habits, sex, race, blood group and age followed by measuring for their risk of leukemia. Furthermore, all major subtypes of leukemia blood samples viz. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML) were collected followed by isolation of mRNA. 8X60K based gene expression profiling using single colour hybridization was performed for the 16 leukemia and 2 normal samples (total 18 collected samples) by Microarray analysis. Up and down regulation of genes with respect to the normal samples has been obtained from total 50, 238 probes covering 14,992 genes.

Results and Discussion:

The leukemia patients were categorized based upon their endogamous marriage system, blood group and smoking habits. Further, 200 genes were sorted out to get highly down regulated or highly up regulated genes with different suitable measure of fold values to verify the expression of already known genes or to distinguish the expression of unknown genes with probable responsible role among this leukemia population. These 200 genes would be further sorted out to determine the highly suppressive or expressive genes and would be formalised with sufficient leukemia samples by Real time PCR based upon double-stranded DNA binding reporters. It would determine the candidate genes which lead to identification of specific biomarkers for different subtypes of Leukemia and designing of Q-PCR based probes to ascertain early diagnosis.

Key words: Leukemia, Epidemiology, Single colour hybridization, probes, Microarray.

Introduction:

Characterization of adrenal adenoma is an important A fundamental step in caring for the patients is to estimate the current burden of blood cancer in India and to understand how the occurrence and outcome of the disease varies across the whole country. In this context, this study aims to describe the prevalence and risk of Leukemia in the population of North Karnataka comprising 14 districts (Figure 1) which covers 3.5% geographical area of India and 2.52% of the whole population according to the Population Census of India, 2001. According to the Leukemia and Lymphoma society, USA, there were approximately 13,410 new cases of AML, 5,200 new cases of ALL, 4570 cases of CML and 15,110 cases of CLL diagnosed in the year 2007-2008 in USA. Again, this society has reported

Address Correspondence to:
Dr. Pramod B. Gai., Director & Professor,
Karnataka Institute for DNA Research,
Pavate Nagar, Dharwar-580003
Email id: pramodbgai@gmail.com, harendra.ind@gmail.com

in the year 2010-11 that blood cancers would account for 9.0 percent of the 1,529,560 new cancer cases diagnosed in the US this year. Leukemia alone comprises 27.5% of cancers affecting the children aged 0 to 19 years in United States. It further states that every 4 minutes, one person in the United States is diagnosed with a blood cancer.[1] The second largest contributor to mortality from childhood cancer in Britain is Leukemia, whereas in India, Leukemia continues to be the largest contributor to cancer related mortality in children (Arora et al, 2009).[2]

Materials and Methods

The medical records of all cases of Leukemia diagnosed at different hospitals and clinics of Dharwad and a major hospital- Karnataka Cancer Treatment and Research Institute (KCTRI), from 2001-2010 were traced through the computer and personal database. From a clinical viewpoint it is desirable to know such factors as the age, sex, blood group, habits and race of patients, because of the influence that these attributes might have upon the care and outcome of the individual case. Different types of leukemia samples were collected followed by mRNA isolation. Single colour hybridization with 8X60K Micro array based gene expression profiling was performed for the 18 samples (16 leukemia samples + 2 normal samples). The microarray data of genes showing up regulation and down regulation with respect to the normal samples have been obtained from total 50, 238 probes covering 14,992 genes. For up regulation, the fold change should be >1(<-1) in the individual treated samples and flag should be detected in the treated samples and can be either detected or compromised in the control. To analyse the expression of unknown genes with probable responsible role or to verify the expression of known genes among leukemia population[3], approximately 200 genes were sorted out to get highly upregulated or highly down regulated genes with different measure of fold values.

Result:

The data obtained during the present investigation are presented in Table 1,2and 3.

Out of 659 eligible cases, data of only 647 patients comprising 230 female and 417 male (overall F:M ratio 1:1.8) have been obtained as reports were missing for 12 patients. The data was first divided religion wise i.e. Hindu and others. Male patients in Hindus have more chances of occurrence of leukemia as compared to female patients. But, it was found that the female patients have more chances of occurrence of leukemia than male patients in the patients of other religions. Significant risk values were observed in females of other religions (p=0.043) and Hindu males (p=0.033). Figure 2 represents that AML patients are significantly affected from Leukemia (p=0.0090). Figure 3 depicts that there is no relation between smoking and Leukemia cases which refutes a earlier report3 as well as supports a previous report4 which advocates the need of application of microarray technique for better research on leukemia on genetic level. The microarray results assessing the regulation of 14,992 genes (Fig:2) have been categorized for Leukemia subtypes wise (Table:4) and has been again sorted out and finalized into 200 highly expressed genes based on different fold values (Table:5).

Discussion

India being multicultural and multi-ethnic have conserved its gene pool because of the intra caste marriage requirement and caste system. Hindus (including Buddhists, Sikhs, Jains) constitute 85% of the population and 15% religious minorities are comprised of Christians and Muslims. In this study, the data of leukemia comprise 86.5% of Hindus and rest 14-15% for other religions which is in concurrence with Indian population. As observed, all different forms of leukemia show higher risk value in male[4]. There is no obvious explanation for this unexpected observation that females appear to be relatively protected against leukemia cases (Jackson et al., 1999).[5] According to the present study, the female patients of other religions (other than Hindus) show higher risk value for the occurrence of leukemia. The facts behind these results emphasize the need for elaborate and precise molecular and genetic studies of the population. The present study would also help to understand the molecular basis and gene expression profiles of the hematologic malignancies, and to apply this

information to develop novel diagnostic, preventive, and therapeutic strategies, identification of novel mutations and candidate genes will facilitate the early detection of leukemia, which in turn will help the clinicians in the diagnosis of blood related malignancies and providing more specific treatment. The above genes would be sorted out again to find the highly expressive or suppressive genes and would be validated with other leukemia samples by Real time PCR.

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Table1: Religion wise leukemia patients by gender

	Male		Female		Total					
Religion	Patients	Contro	Patients	Control	Patients	Contro	Odds Ratio	95% CI for OR		p-value
Hindu	366	627	194	419	560	1046	1.2607	1.0130	1.5708	0.0333*
Others	51	112	36	45	87	157	0.5692	0.3173	1.0251	0.0433*

Table2: Association between blood groups and different leukemia in male and females

Blood group	CLL		CML		ALL	
	Male	Female	Male	Female	Male	Female
A+ve	8	4	37	16	31	17
B+ve	18	7	35	18	45	26
AB+ve	9	3	9	6	3	1
O+ve	9	4	40	28	54	17
Total	44	18	121	68	133	61
	Chi-square=0.2340 P = 0.9723		Chi-square=1.7680 P = 0.6220		Chi-square=3.1640 P = 0.3670	

Table3: Location vs groups in different leukomia

Types	Smoking	Rural	Urban	Total	Odds Ratio	95% CI for OR		p-value
CLL	Nonsmoker	37	16	53	2.8906	0.5333	16.3204	0.1371
	Smoker	4	5	9				
CML	Nonsmoker	79	60	139				
	Smoker	9	11	20	1.6093	0.5637	4.6875	0.3195
ALL	Nonsmoker	15	9	24				
	Smoker	1	2	3	3.3333	0.1461	209.4819	0.3324
AML	Nonsmoker	74	39	113				
	Smoker	5	7	12	2.6564	0.6697	11.2594	0.1038

Summary of Differentially Regulated Genes :
Table 4 : A huge data of genes showing up regulation and down regulation with respect to the normal samples has been obtained respect to the normal samples has been obtained from total 50, 238 probes

Samples hybridized	Up	Down
ALL	775	251
AML	2306	4088
CLL	4247	4247
CML	863	734

Table 5: To analyze the expression of unknown genes with probable responsible role or to verify the expression of known genes among local leukemia population, approximately 200 genes from 14,992 genes were sorted out further to get highly upregulated or highly down regulated genes with different measure of fold values.

Types	CML	CLL	AML	ALL	Types	CML	CLL	AML	ALL
	Up regulated genes					Down regulated genes			
Fold value	3.8	5.0	4.8	3.3	Fold Value	-2	-3	-3	-2
No. of genes	30	27	23	30	No. of genes	22	24	22	18

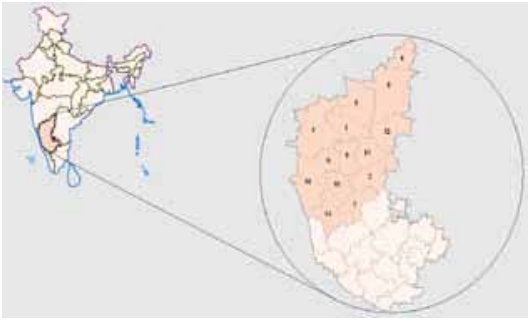

Figure 1. North Karnataka Region. 1, Bagalkot; 2,Bellary; 3, Belgaum; 4, Bidar; 5, Bijapur; 6, Dharwad; 7,Davangere; 8, Gulbarga 9: Gadag; 10, Haveri; 11, Koppal; 12,Raichur; 13, Shimoga; 14, Uttar Kannada

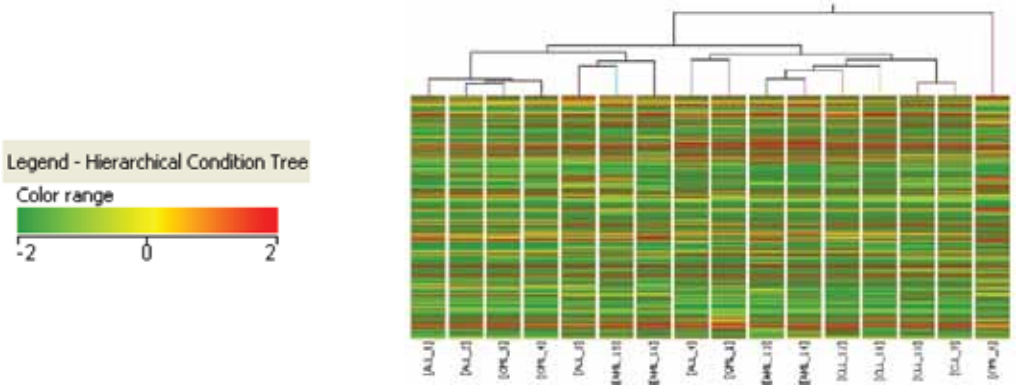

Fig.2: Clusters for Intra array quality control. Normalization has been performed by GeneSpring GX 11.5 Software.

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